EXHIBIT A

IMMUNOLOGY

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W. H. Freeman and Company
New York

Cover illustration of AIDS viruses budding from an infected T cell was provided by L. Montagnier/CNRI, Science Photo Library.

Library of Congress Cataloging-in-Publication Data

Kuby, Janis.
Immunology / Janis Kuby.
p. cm.
Includes bibliographical references and index.
ISBN 0-7167-2257-7
1. Immunology. I. Title.
[DNLM: 1. Immune System. 2. Immunity. QW 504 K95i]
QR181.K83 1992
616.07'9—dc20
DNLM/DLC 91-44429
for Library of Congress CIP

Printed in the United States of America

3 4 5 6 7 8 9 RRD 9 9 8 7 6 5 4 3

sosomes are derived from the golgi complex and contain large numbers of degradative enzymes, including proteases, nucleases, lipases and other hydrolytic enzymes. Within secondary lysosomes, the ingested macromolecules are then digested into small breakdown products (e.g., peptides, nucleotides, and sugars) in the *endosomal processing pathway.*

Phagocytosis involves the ingestion of particulate material, including whole pathogenic microorganisms (Figure 1-4). In phagocytosis, which differs from endocytosis in several ways, the plasma membrane expands around the particulate material to form large vesicles called *phagosomes.* These vesicles are roughly 10-to-20 times larger than endocytic vesicles. The expansion of the membrane in phagocytosis requires participation of microfilaments, which do not take part in endocytosis. Only specialized cells are capable of phagocytosis, whereas endocytosis is carried out by virtually all cells. The specialized phagocytic cells include blood monocytes, neutrophils, and tissue macrophages (see Chapter 3). Once particulate material is ingested into phagosomes, the phagosomes fuse with lysosomes and the ingested material is then digested in the endosomal processing pathway by a process similar to that seen in endocytosis.

Barriers Created by the Inflammatory Response

Tissue damage caused by a wound or by invasion by a pathogenic microorganism induces a complex sequence of events collectively known as the *inflammatory response.* Many of the classic features of the inflammatory response were described as early as 1600 B.C. in Egyptian papyrus writings. In the first century A.D., the Roman physician Celsus described the "four cardinal signs of inflammation" as *rubor* (redness), *tumor* (swelling), *calor* (heat), and *dolor* (pain). In the second century A.D., another physician, Galen, added a fifth cardinal sign: *functio laesa* (loss of function).

The cardinal signs of inflammation reflect three major events that occur in an inflammatory response: (1) increased blood flow, (2) increased capillary permeability, and (3) influx of phagocytic cells. The increase in blood flow to the affected area occurs as the blood vessels that carry blood away from the area constrict, resulting in engorgement of the capillary network. The engorged capillaries produce tissue redness (*erythema*) and an increase in tissue temperature. An increase in capillary permeability facilitates an influx of fluid and cells from the engorged capillaries into the surrounding tissue. The fluid that accumulates (*exudate*) has a much higher protein content than fluid normally released from the vasculature. Accumulation of exudate contributes to the tissue swelling. The increased capillary permeability also

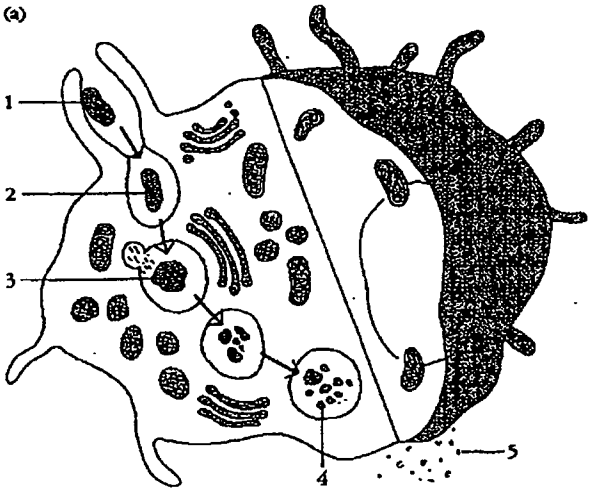


Figure 1-4 (a) The steps in phagocytosis: 1) attachment of the bacteria to long membrane evaginations, called pseudopodia, 2) ingestion into phagosomes, movement of the phagosome toward the lysosome, 3) fusion of the lysosome with the phagosome, releasing the enzyme contents of the lysosome into the phagosome, 4) digestion and 5) elimination of the ingested material. (b) Transmission electron micrograph of a phagocytic cell (neutrophil) showing pseudopods engulfing *E. coli* bacteria. (Calibration mark = 1 micron.) [Part (b) courtesy of Dr. Joseph R. Goodman, Dept. of Pediatrics, University of California at San Francisco.]

facilitates the migration of various white blood cells from the capillaries into the tissues. Phagocytic cells are the major type of white blood cell to emigrate. The emigration of phagocytes involves several steps: first, the phagocytes adhere to the endothelial wall (*margination*); then, they move between the capillary endothelial cells into the tissue (*diapedesis*); and finally, they migrate through the tissue to the site of the wound or infection (*chemotaxis*) (Figure 1-5).

The events in the inflammatory response are initiated by a complex series of interactions involving several chemical mediators; whose interactions are still only partially understood. Some of these mediators are derived from invading microorganisms, some are released from damaged tissue, some are generated by several plasma enzyme systems, and some are products of various white blood cells participating in the inflammatory response. Among the chemical mediators released in response to tissue damage are various serum proteins called *acute-phase proteins*. The concentrations of these proteins increase dramatically in tissue-damaging infections. *C-reactive protein* is a major acute-phase protein produced by the liver in response to tissue damage. C-reactive protein binds to the C-polysaccharide cell-wall component found on a variety of bacteria and fungi and activates the complement system, resulting in increased clearance of the pathogen either by complement-mediated lysis of the pathogen or by complement-mediated increases in phagocytosis.

Four enzyme systems in blood plasma participate in the inflammatory response: the clotting system, the kinin system, the fibrinolytic system, and the complement system. These four enzyme systems generate factors that induce constriction of the blood vessels, increased capillary permeability, diapedesis, chemotaxis, and clearance of the pathogen. These systems are discussed in more detail in Chapter 11.

Acquired (Specific) Immunity

Acquired, or specific; immunity reflects the presence of a functional immune system that is capable of specifically recognizing and selectively eliminating foreign micro-

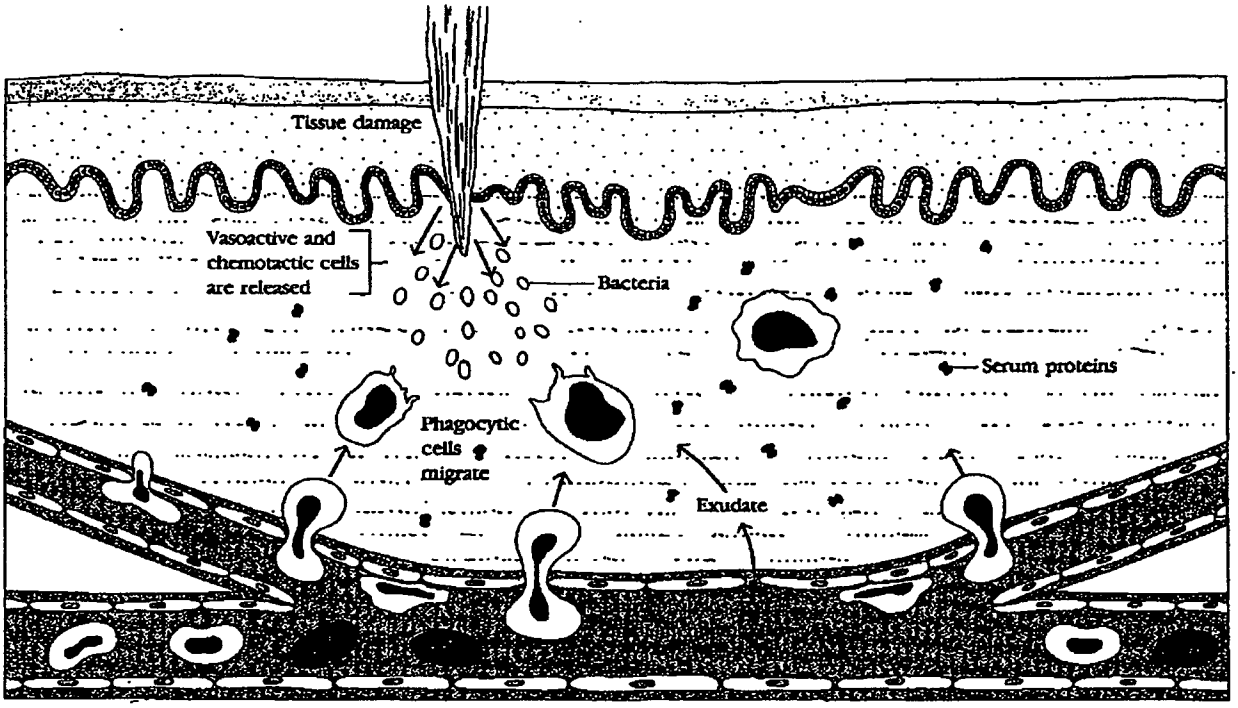


Figure 1-5 The inflammatory response. A bacterial infection causes tissue damage with release of various vasoactive and chemotactic factors. These factors induce increased blood flow to the area, increased capillary permeability, and influxes of white blood cells, including phagocytic and lymphocytes, from the blood into the tissues. The serum proteins contained in the exudate have anti-bacterial properties and the phagocyte will begin to engulf the bacteria.

Role of Cytokines in the Inflammatory Response

In response to infection or tissue injury, a complex cascade of nonspecific events, known as the *acute-phase response* (*APR*), is initiated that provides early protection by restricting the tissue damage to the site of infection or tissue injury. The acute-phase response involves both localized and systemic responses. The localized inflammatory response develops as plasma clotting factors are produced resulting in activation of the clotting, kinin-forming, and fibrinolytic pathways. Various cytokines have been shown to influence this localized inflammatory response by facilitating both the adherence of inflammatory cells to vascular endothelial cells and their migration through the vessel and into the tissue spaces. This results in an influx of lymphocytes, neutrophils, monocytes, eosinophils, basophils, and mast cells to the site of tissue damage, where these cells participate in clearance of the antigen.

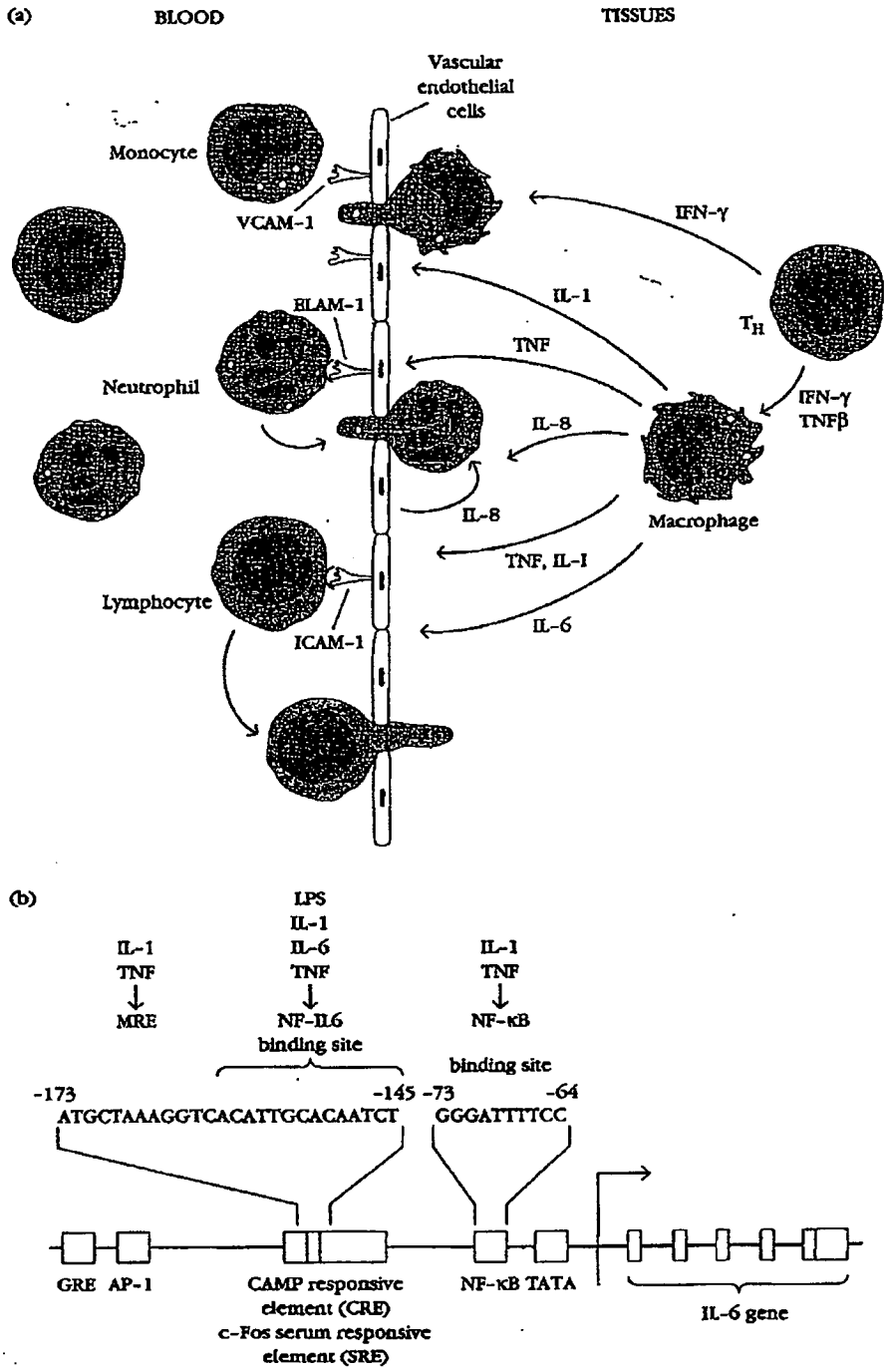
The systemic response includes the induction of fever, increased synthesis of hormones such as ACTH and hydrocortisone, increased white blood cell production (leukocytosis), and production of a large number of hepatocyte-derived acute-phase proteins including C-reactive protein (CRP) and serum amyloid A (SAA). The increase in body temperature inhibits the growth of a number of pathogens and appears to enhance the immune response to the pathogen. C-reactive protein (CRP) is a prototype acute-phase protein whose serum levels increase by 1000-fold during an acute-phase response. It is composed of five identical polypeptides held together by noncovalent interactions. C-reactive protein binds to a wide variety of microorganisms and activates complement, resulting in the deposition of a complement component, C3b, on the surface of the microorganism. Phagocytic cells express C3b receptors and phagocytose the C3b-coated microorganisms.

The acute-phase inflammatory reaction is initiated following the activation of tissue macrophages and the release of three cytokines: TNF-α, IL-1, and IL-6. These three cytokines act synergistically to induce many of the localized and systemic changes observed in the acute-phase inflammatory response. All three cytokines act locally on fibroblasts and endothelial cells inducing coagulation and an increase in vascular permeability. Both TNF and IL-1 induce increased expression of adhesion molecules on vascular endothelial cells. TNF has been shown to induce increased expression of ELAM-1, an endothelial leukocyte adhesion molecule that selectively binds neutrophils. IL-1 induces increased expression of ICAM-1 and VCAM-1, the intercellular adhesion molecules for lymphocytes and monocytes. Circulating neutrophils, monocytes, and lymphocytes adhere to the wall of a blood vessel by recognizing these adhesion molecules and then move through the vessel wall into the tissue spaces (Figure 11-10a). IL-1 and TNF also act on macrophages and endothelial cells inducing production of IL-8. IL-8 contributes to the influx of neutrophils by increasing their adhesion to vascular endothelial cells and by acting as a potent chemotactic factor. Other cytokines also serve as chemotactic factors for various leukocyte populations. For example, IFN-γ has been shown to chemotactically attract macrophages, bringing increased numbers of phagocytic cells to a site where antigen is localized. In addition, IFN-γ and TNF activate macrophages and neutrophils, promoting increased phagocytic activity and increased release of lytic enzymes into the tissue spaces.

The combined action of IL-1, TNF, and IL-6 are also responsible for many of the systemic changes that occur during an acute-phase inflammatory response. Each of these cytokines acts on the hypothalamus to induce a fever response. Within 12–24 h of an acute-phase inflammatory response, increased levels of IL-1, TNF, and IL-6 induce hepatocyte production of acute-phase proteins. TNF also acts on vascular endothelial cells and macrophages inducing secretion of colony-stimulating factors (M-CSF, G-CSF, and GM-CSF). Production of CSFs will result in induction of hematopoiesis, resulting in transient increases in the necessary white blood cells to fight the infection.

TNF, IL-1, and IL-6 are not produced constitutively by cells. Instead synthesis of these cytokines is induced by various stimuli including certain viruses, the endotoxin component of gram-negative bacterial cell walls, as well as the cytokines themselves. Both TNF and IL-1 have been shown to induce expression of each other as well

Figure 11-10 The acute-phase inflammatory response is mediated by three principal cytokines: TNF-α, IL-1, and IL-6. (a) These three cytokines act synergistically to induce many of the events leading to an inflammatory response including increased adherence of circulating white blood cells to vascular endothelial cells and their extravasation to the tissue spaces. IL-1 induces increased expression of ICAMs on the vascular endothelial cells. IL-8 contributes to the process by inducing neutrophil adherence to the endothelial cells. IL-8 and IFN-γ exhibit chemotactic activity for neutrophils and macrophages, respectively. IFN-γ also functions to activate macrophages, increasing their phagocytotic ability and their lysosomal enzyme content. (b) TNF-α, IL-1, and IL-6 have been shown to be induced together. The molecular mechanism that coordinates the expression of these three cytokines is begining to be unraveled. In the case of the IL-6 gene, for example, both IL-1 and TNF-α have been shown to induce the expression of DNA-binding proteins that bind to the IL-6 enhancer, resulting in activation of the IL-6 gene. Thus, as IL-1 and TNF-α levels increase, IL-6 production is also increased.


(a)
BLOOD
TISSUES
Vascular endothelial cells
Monocyte
VCAM-1
IFN-γ
IL-1
ELAM-1
T_H
TNF
Neutrophil
IFN-γ
TNFβ
IL-8
IL-8
Macrophage
TNF, IL-1
Lymphocyte
IL-6
ICAM-1
(b)
LPS
IL-1
IL-6
TNF
IL-1
TNF
IL-1
TNF
MRE
NF-IL6 binding site
NF-κB binding site
-173
-145
-73
-64
ATGCTAAAGGTCACATTGCACAATCT
GGGATTTTCC
GRE
AP-1
CAMP responsive element (CRE)
c-Fos serum responsive element (SRE)
NF-κB
TATA
IL-6 gene

as expression of IL-6. The genes for TNF, IL-1, and IL-6 have been cloned, and nuclear factors that bind to promoter or enhancer sequences are beginning to be identified. In the case of the IL-6 gene, for example, the promoter sequence has been shown to contain several regulatory regions to which DNA-binding proteins bind (Figure 11-10b). Three of these DNA-binding proteins are the nuclear factor IL-6 (NF-IL6), the multiresponse element (MRE), and nuclear factor κB (NF-κB). All three DNA binding proteins have been shown to be induced by IL-1 and by TNF. Thus, as IL-1 or TNF levels increase, IL-6 production is also increased.

It is important that the duration and intensity of the inflammatory response be carefully regulated to control tissue damage and facilitate the tissue repair mechanisms that are necessary for wound healing. TGF-β has been shown to play an important role in limiting the inflammatory response. It also promotes accumulation and proliferation of fibroblasts and the deposition of an extracellular matrix which is required for proper tissue repair.

Cytokines and Disease

Defects in the complex regulatory networks governing the expression of cytokines and cytokine receptors have been implicated in a number of diseases. Overexpression or underexpression of an appropriate or inappropriate cytokine or cytokine receptor may contribute to a disease process. In this section, several examples of diseases resulting from cytokine abnormalities are described and the possible therapeutic uses of cytokines are discussed.

Bacterial Septic Shock

The role of cytokine overproduction in pathogenesis can be illustrated by bacterial septic shock. This condition may develop within a few hours following infection by certain gram-negative bacteria including *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter aerogenes,* and *Neisseria meningitidis.* The symptoms of bacterial septic shock, which often is fatal, include a drop in blood pressure, fever, diarrhea, and widespread blood clotting in various organs. The incidence of shock with gram-negative bacterial sepsis is quite high, and has been estimated to develop in 5 out of every 1000 patients admitted to hospitals. The mortality rate is also high and treatment with conventional antibiotics is of little benefit.

Bacterial septic shock appears to develop when bacterial cell-wall endotoxins stimulate macrophages to overproduce IL-1 and TNF-α. It is the increased levels of IL-1 and TNF-α that cause septic shock. In one study, for example, higher levels of TNF-α were found in patients who died of meningitis than in those who recovered. Furthermore, a condition resembling bacterial septic shock can be produced by injection of recombinant TNF-α in the absence of gram-negative bacterial infection. Recent reports offer some hope that neutralization of TNF-α or IL-1 activity with monoclonal antibodies or antagonists may prevent this fatal shock from developing in these bacterial infections. Monoclonal antibody to TNF-α was shown to prevent an otherwise fatal dose of endotoxin-induced shock in animal models. And another study has shown that injection of a recombinant IL-1 receptor antagonist, which can prevent IL-1 binding to the IL-1 receptor, significantly reduced the mortality due to septic shock in rabbits. It is hoped that these experimental results will have therapeutic benefit for the treatment of bacterial septic shock in humans.

Bacterial Toxic Shock and Related Diseases

A variety of microorganisms produce toxins that act as superantigens, stimulating large numbers of T cells irrespective of their antigenic specificity. As discussed in Chapter 4, superantigens bind simultaneously to a class II MHC molecule and to the V_β region of the T-cell receptor, activating all T cells bearing a particular V_β family (see Figure 4-16). Unlike conventional antigens, superantigens are not internalized, processed, and presented by antigen-presenting cells. Instead they bind directly to the class II MHC molecule, apparently binding outside of the antigen-binding cleft of the MHC molecule. Once the superantigen is bound to the class II MHC molecule, it binds to a particular part of the T-cell receptor V_β chain. Unlike the T-cell response to conventional antigens which is MHC restricted, T cells can be activated by superantigens bound to allogeneic or even xenogeneic MHC molecules. Thus, superantigens appear to violate the basic tenet of MHC restriction for T-cell activation. The interaction of the superantigen with the T-cell receptor appears to involve regions of the V_β chain that are well away from the complementarity-determining regions of the TCR, suggesting that the superantigen interacts with a site that is distinct from the conventional antigen–MHC binding site on the TCR. The superantigen is thought to bind to a region of β pleated sheet exposed on the side of the TCR. Superantigens activate large numbers of T cells. While only